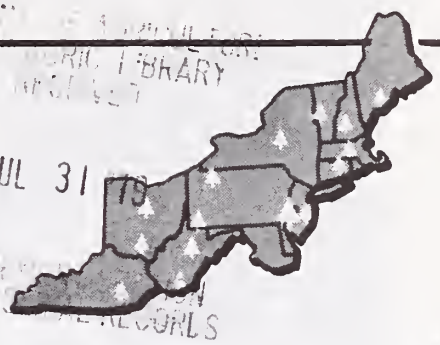
FOREST SERVICE RESEARCH NOTE NE-281
1979



Northeastern Forest Experiment Station

FOREST SERVICE, U.S. DEPARTMENT OF AGRICULTURE, 370 REED ROAD, BROOMALL, PA. 19008

A SIMPLE TECHNIQUE FOR COLLECTING CHYLE FROM THE GYPSY MOTH, *Lymantria dispar* L.

—FRANK S. KACZMAREK
University of Connecticut, Farmington

—NORMAND R. DUBOIS
Microbiologist
Northeastern Forest Experiment Station,
Hamden, Conn.

Abstract. A procedure for rapidly obtaining significant quantities of chyle is described. The amount and composition of chyle collected from larvae of the gypsy moth, *Lymantria dispar* (L.), varied according to the instar examined and the age within the instar.

In our studies on the effect of gypsy moth (*Lymantria dispar* L.) chyle (digestive juices) on the nucleopolyhedrosis virus, several techniques commonly used to obtain the chyle from insects were unsatisfactory. Such techniques, including dissection and maceration of the midgut of the insect (Turunen and Chippendale 1977; Baker 1976; Ahmad et al. 1976), and electroshock (Aizawa 1962; Euguchi and Iwamoto 1976) have inherent drawbacks. When using the midgut dissection procedures, no differentiation is made between intracellular and extracellular material collected. The electroshock method works well but it is time consuming. We resolved this problem by developing a simple procedure for quickly obtaining sizeable quantities of chyle free of cellular debris from various instars and larval sizes.

MATERIALS AND METHODS

Gypsy moth larvae were reared on an artificial diet (ODell and Rollinson 1966) and maintained in an environmental chamber at 26 to 27°C with a 12:12 DL photoperiod. The larvae were reared in 0.47-liter (pint) cardboard containers with plastic lids. Ten larvae were reared in each container, and diet was supplied every 48 h.

Chyle was obtained by grasping each larva with a pair of forceps at approximately the third segment from the posterior end, and turning the larva so that it lay dorsally along the length of the forceps. The larva was forced to regurgitate after gentle pressure was applied with the side of a micropipette along the ventral side of the insect. The drop of chyle accumulating at the mouth was then

Figure 1.—Chyle collection from a 5th-instar larva.

collected with the micropipette (Fig. 1). Occasionally too much pressure was applied and gut wall cells were regurgitated with the chyle; those chyle samples and the larvae were discarded. The chyle was pooled in small 5-ml test tubes chilled in an ice bath. A few crystals of phenyl thiourea (PTU) were added to prevent melanization. The chyle was then frozen at −20°C until needed.

The pH of the pooled chyle was estimated with narrow range pH paper (Micro Essential Laboratory, Brooklyn, N.Y.).[1] Protein determinations were made by the Lowry procedure (Lowry et al. 1951), using bovine serum albumin as a standard.

RESULTS AND CONCLUSION

Table 1 summarizes the chemical and physical characteristics of chyle obtained from three instars by our technique. The average pH and color of the chyle did not differ appreciably from instar to instar. However, there was a significant difference in the amount of chyle obtained from each instar examined. Noteworthy was the observation that the protein concentration decreased with the increasing age of the larvae. As expected, the largest volume of chyle was collected from the large 5th-instar larvae. Within each instar, the

[1]The use of trade, firm or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.

Table 1.—Characteristics of the chyle obtained from various instars of the gypsy moth

Instar examined	Number larvae pooled	Average volume of[a] chyle/larva	Average weight of larvae	Estimated chyle pH	Protein concentration in chyle	Description of chyle	Particulate[b] matter present
		(ul)	*(mg)*		*(ug/ul)*		
3rd instar							
Day 0	10	1.05	22.28	9.3-9.5	89.50	faint brown	−
Day 2	10	0.15	45.47	9.2-9.3	400.00	cloudy brown	+ +
Day 4	10	0.55	82.17	9.2-9.4	143.01	light brown	+
4th instar							
Day 0	8	2.50	55.25	9.3-9.4	43.27	faint brown	−
Day 2	10	2.15	153.25	9.0-9.2	65.15	yellowish brown	+ +
Day 4	10	0.95	260.60	8.2-8.3	175.25	dark brown	+ +
5th instar							
Day 0	7	13.6	238.91	9.0-9.5	28.67	faint brown	−
Day 2	10	1.7	391.20	8.9-9.0	68.32	turbid yellow	+ +
Day 4	10	2.0	624.11	8.1-8.4	66.66	yellowish brown	+ +

[a]Refers to the amount of digestive fluid regurgitated by the larvae and is not an indication of the total amount of digestive fluid present in each larvae.
[b]− = clear; + = slight amount; + + = heavy amount.

largest collection was obtained right after the larva molted and just before feeding resumed. The chyle collected during this stage of instar development was always free of contaminating particulate matter (usually partially digested food) compared with chyle collected at other times within the same instar.

For gypsy moth larvae, the time at which the chyle is taken is critical. Our technique facilitates the acquisition of large quantities of chyle with minimal expenditure of time and effort. Though the larvae used in this study was forced to regurgitate only once, we found that in using this technique, larvae could be forced to regurgitate every 3 or 4 days without apparent ill effects. However, more frequent forced regurgitations often resulted in reduced feeding and death.

LITERATURE CITED

Ahmad, Z., M. Saleemuddin, and M. Siddiqi.
1976. **Alkaline protease in the larvae of the army worm,** *Spodoptera litura*. Insect Biochem. 6:501-505.

Aizawa, K.
1962. **Antiviral substance in the gut juice of the silkworm,** *Bombyx mori* **(L.).** J. Insect Pathol. 4:72-76.

Baker, J. E.
1976. **Properties of midgut proteases in larvae of** *Attagenus megatoma*. Insect Biochem. 6:143-148.

Euguchi, M., and A. Iwamoto.
1976. **Alkaline proteases in the midgut tissue and digestive fluid of the silkworm,** *Bombyx mori*. Insect Biochem. 6:491-496.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. S. Randall.
1951. **Protein measurement with the folin phenol reagent.** J. Biol. Chem. 193:265-275.

ODell, T. M., and W. D. Rollinson.
1966. **A technique for rearing the gypsy moth,** *Porthetria dispar* **(L.) on an artificial diet.** J. Econ. Entomol. 59:741-742.

Turunen, S., and G. M. Chippendale.
1977. **Esterase and lipase activity on the midgut of** *Diatracea grandiosella*: **digestive functions and distribution.** Insect Biochem. 7:67-71.

ACKNOWLEDGMENT

The authors wish to express appreciation to R. T. Zerillo for his photographic assistance. This study was supported by the USDA Gypsy Moth Research, Development and Application Program.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1979—603-011/31

MANUSCRIPT RECEIVED FOR PUBLICATION 5 MARCH 1979